0.75=Average total column porosity; and
F=Flow rate in milliliters per minute.

The capacity factor (*k'*) for minocycline is satisfactory if it is not less than 6.2 and not more than 11.5.

If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(*b*) of this section should not be changed.

(iv) *Calculations*—Calculate the micrograms of minocycline per milligram of sample as follows:

$$\begin{array}{c} \text{Micrograms of} \\ \text{minocycline} \\ \text{per milligram} \end{array} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

A_u=Area of the minocycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);
A_s=Area of the minocycline peak in the chromatogram of the minocycline working standard;
P_s=Minocycline activity in the minocycline working standard solution in micrograms per milliliter;
C_u=Milligrams of minocycline sample per milliliter of sample solution; and
m=Percent moisture content of the sample.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of minocycline per milliliter.

(5) *Epi-minocycline content.* Proceed as directed in paragraph (b)(1) of this section. Calculate the epi-minocycline content as follows:

$$\text{Percent Epi-minocycline} = \frac{(A_{epi}) \times 100}{(A_{total})}$$

A_{epi}=Area of the epi-minocycline peak in the chromatogram of the sample; and
A_{total}=The sum of the areas of all the peaks eluting after the solvent front.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.5 percent potassium bromide disc prepared

as described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(8) *Residue on ignition.* Proceed as directed in § 436.207(b) of this chapter.

(9) *Absorptivity.* Accurately weigh about 1 gram of sample into a 100-milliliter volumetric flask, dissolve, and dilute to mark with deionized water. Determine the absorbance of this solution on a suitable spectrophotometer at 560 nanometers (nm) using 5-centimeter cells with water in the reference cell. Calculate the absorptivity as follows:

$$\begin{array}{c} \text{Absorptivity at 560} \\ \text{nm} \end{array} = \frac{(A_{560})\,(100)}{\begin{array}{c}\text{(grams of sample)}\\ (1,000)(5)\end{array}}$$

[39 FR 19076, May 30, 1974, as amended at 43 FR 11156, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978; 44 FR 22058, Apr. 13, 1979; 50 FR 19920, May 13, 1985; 53 FR 32607, Aug. 26, 1988; 53 FR 39839, Oct. 12, 1988; 54 FR 47205, Nov. 13, 1989]

§ 446.65 Oxytetracycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline is [4S-(4α,4aα,5α,5aα,6β,12aα)]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octa - hydro- 3,5,6,10,12,12a-hexahydroxy-6-methyl- 1,11-dioxo-2-naphthacenecar - boxamide dihydrate. Oxytetracycline is produced by the growth of *Streptomyces rimosus*. It is so purified and dried that:

(i) Its potency is not less than 832 micrograms of oxytetracycline per milligram on an ''as is'' basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 6 percent and not more than 9 percent.

(iv) Its pH in an aqueous suspension containing 10 milligrams per milliliter is not less than 4.5 and not more than 7.0.

(v) When calculated on an anhydrous basis its absorptivity at 353 nanometers relative to that of the oxytetracycline working standard similarly treated is 100±4 percent.

(vi) It gives a positive result to an identity test for oxytetracycline.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—*(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Chemical assay.* Proceed as directed in §436.320 of this chapter.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous suspension containing 10 milligrams per milliliter.

(5) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 50 milligrams each of the sample and standard in 250 milliliters of 0.1*N* hydrochloric acid. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with 0.1*N* hydrochloric acid. Using a suitable spectrophotometer and 0.1*N* hydrochloric acid as the blank, determine the absorbance of each solution at 353 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{Milligrams of standard}}{\text{Absorbance of standard} \times \text{Milligrams of sample}} \times \text{Potency of standard in micrograms per milligram} \times \frac{10}{100 - m}$$

where: *m* = Percent moisture in the sample.

(6) *Identity.* To about 1 milligram of sample, add 2 milliliters of sulfuric acid; a light-red color is produced when oxytetracycline is present.

(7) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[43 FR 11156, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.65a Sterile oxytetracycline.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Sterile oxytetracycline is [4S - (4α,4aα,5α,5aα,6β,12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11, 12a - octahydro - 3,5,6,10,12,12a - hexahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide dihydrate. Oxytetracycline is produced by the growth of *Streptomyces rimosus.* It is so purified and dried that:

(i) Its potency is not less than 832 micrograms of oxytetracycline per milligram on an ''as is'' basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its moisture content is not less than 6 percent and not more than 9 percent.

(vii) Its pH in an aqueous suspension containing 10 milligrams per milliliter is not less than 4.5 and not more than 7.0.

(viii) When calculated on an anhydrous basis, its absorptivity at 353 nanometers relative to that of the oxytet- racycline working standard similarly treated, is 100±4 percent.

(ix) It gives a positive result to an identity test for oxytetracycline.

(x) It is crystalline.